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■原　著■

Seroprevalence of anti-HEV and epidemiological features in hog raising area and non hog raising area

Kazuyoshi Ishikawa *1, Junko Sasaki *2, Akio Miyasaka *3, Yukiho Kasai *3, Ichiro Kumagai *3, Shigehiko Sainokami *4, Koichi Abe *3, Yasuhiro Takikawa *3, Akinobu Kato *3, Kazuyuki Suzuki *3

Abstract

We randomly selected 395 residents from hog raising area and 379 residents from non hog raising area and determined anti-HEV in their sera using EIA kit (Cosmic Corporation). Anti-HEV was detected in 6.8% in hog raising area and in 4.2% in non hog raising area respectively. It was found out that the frequency of anti-HEV tended to be higher in hog raising area compared with non hog raising area (p=0.113).The average age and ALT level in residents positive and negative for anti-HEV in hog raising area were 59.3±9.8 vs. 59.3±10.1 yrs., 30.1±12.8 vs. 25.3±18.9 IU/L, and those in non hog raising area were 58.7±10.7 vs. 60.6±9.6 yrs., 31.5±23.3 vs. 24.9±13.6 IU/L, respectively. The average ALT level in residents positive for anti-HEV tended to be higher compared with that in those negative for anti-HEV in non hog raising area (p=0.069). Anti-HEV positive rate according to age showed a tendency to increase with aging in hog raising area and to decrease with aging in non hog raising area. Therefore anti-HEV positive rate in 60's was significantly higher in hog raising area compared with non hog raising area (8.9% vs. 3.3%, $p < 0.05$). Moreover anti-HEV titer distributed commonly in lower range in hog raising area while it showed bimodal distribution in lower and higher range in non hog raising area. These results demonstrate that there may be a difference in the route of HEV infection as a community acquired disease between hog raising area and non hog raising area.

Key words：seroprevalence, anti-HEV, swine, hog raising area, raw meat

Introduction

Recently, hepatitis E virus (HEV) infection in Japan has been considered to be a zoonosis, which spreads from swine, wild boars or deer to humans being infected with an indigenous strain via a food-borne mode in the community. We reported that among blood donors 3.5% were positive for anti-HEV IgG, and they had a tendency to be older than those negative for anti-HEV, possibly just reflecting longer exposure to the environment. Furthermore those positive donors had no recognized symptoms that implied the presence of the disease in the past suggesting that subclinical infection of HEV appears to occur more frequently than apparent infection1). Therefore it is important to clarify the epidemiological status of HEV infection in the residents of hog raising area and non-hog raising area in the region such as X prefecture where a lot of hoggery is present to elucidate the mode of HEV transmission as a community acquired disease.

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*1 Faculty of Nursing, Iwate Prefectural University
*2 Iwate Health Service Association
*3 First Department of Internal Medicine, Iwate Medical University
*4 Department of Liver Disease, Mizusawa City Hospital

Introduction

Recently, hepatitis E virus (HEV) infection in Japan has been considered to be a zoonosis, which spreads from swine, wild boars or deer to humans being infected with an indigenous strain via a food-borne mode in the community. We reported that among blood donors 3.5% were positive for anti-HEV IgG, and they had a tendency to be older than those negative for anti-HEV, possibly just reflecting longer exposure to the environment. Furthermore those positive donors had no recognized symptoms that implied the presence of the disease in the past suggesting that subclinical infection of HEV appears to occur more frequently than apparent infection1). Therefore it is important to clarify the epidemiological status of HEV infection in the residents of hog raising area and non-hog raising area in the region such as X prefecture where a lot of hoggery is present to elucidate the mode of HEV transmission as a community acquired disease.

Materials and method

Objects

We randomly selected 395 residents from 4 hog raising areas and 379 residents from 6 non hog raising areas in X Prefecture during the period between June 2005 and October 2005. Their average age and male/female ratio were 59.3±10.0 years and 131：264 and 60.6 ±9.6 years and 132：247, respectively. Here we defined a hog raising area as the town or village where swineries are present and the number of the breeding pigs is more than 20,000 and non hog raising area as the town or village where no swineries are present

Determination of anti-HEV

Anti-HEV IgG was determined using the VIRAGENT HEV-Ab kit (Cosmic Corporation Co. Ltd., Tokyo, Japan), employing an enzyme-linked immunosorbent assay. Briefly, 50 μl of a serum specimen, which was diluted 101 times with diluent was added to a plate coated with HEV antigen together with 50 μl of positive and negative control. The reactive reagent was then added for incubation for 1 hour at room temperature. The reactive reagent was removed by washing and then 50μl of enzyme labeled antibody was added to the reactive reagent for incubation for 1 hour at room temperature. After washing, 50μl of reactive enzyme for color development was added and the plate left for 10 minute in the dark at room temperature. Next, 50μl of a quenching liquid was added to stop the reaction and then absorbance was measured at 450 nm using a plate reader. Any specimen at or over the cut-off threshold of 13 was judged to be positive for anti-HEV IgG.

Serum transferases and total cholesterol

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT) ant total cholesterol (TC) values also were determined.

Statistical analysis

Statistical analysis was performed using SPSS 14.0J for Windows (SPSS Inc., IL, USA). Fischer's exact probability test and Student's t-test were used to determine significant differences, and P values of less than 5% were considered significant.

Ethical considerations

The present study conformed to the guidelines of epidemiological studies devised by the Ministry of Health, Labor and Welfare, Japan, to prevent leakage of personal information, and were conducted to for the purpose of social profit.

Result

1) Positive rate of ant-HEV (Table 1,2)

Anti-HEV was detected in 6.8% in hog

raising area and in 4.2% in non hog raising area, respectively. It was found out that the frequency of anti-HEV tended to be higher in hog raising area compared with non hog raising area ($p=0.113$), however, the sex differences were not found in both areas.

2) Age distribution of anti-HEV on hog raising area and non hog raising area (Figure 1)

Anti-HEV positive rate according to age showed a tendency to increase with aging in hog raising area and to decrease with aging in non hog raising area. Therefore anti-HEV positive rate in 60' s was significantly higher in hog raising area compared with that in non hog raising area (8.9% vs. 3.3%, $p < 0.05$).

3) Distribution of anti-HEV titers in hog raising area and non hog raising area (Figure 2) Anti-HEV titer distributed more commonly in lower range in hog raising area while it showed bimodal distribution in lower and higher range in non hog raising area.

4) Comparison of age and laboratory data between anti-HEV positive and negative residents in hog raising (Table 3) and non hog raising area (Table 4)

The average GGT value in residents positive for anti-HEV was significantly higher compared with that in those negative for anti-HEV in hog arising area ($p<0.05$). The average ALT and AST values in residents positive for anti-HEV tended to be higher and TC value to be lower compared with those in residents negative for anti-HEV in non hog raising area ($p<0.1$).

Discussion

Until recently, in Japan HEV infection has been recognized as the imported hepatitis

Table 1. The positive rate of anti-HEV

Area	No. positive(%)		No. measured
Hog raising	27 (6.8*)	†	395
Non hog raising	16 (4.2**)	†	379
Total	43 (5.9)		774

*95%CI(4.3-9.3), **95%CI(2.2-6.2) †p=0.113

Table 2. The positive rate of anti-HEV in males and females

Area	No. +ve	male(%) / female(%)
Hog raising	27	10 (7.6) / 17 (6.4)
Non hog raising	16	7 (5.3) / 9 (3.6)
Total	43	17 (6.5) /26 (5.1)

Table 3. Comparison of age and laboratory data between anti-HEV positive and negative residents in hog raising area

	positive (N=27)	negative(N=368)
Age	59.3±9.8	59.3±10.1
AST(IU/L)	28.1±8.0	24.9±12.0
ALT (IU/L)	30.1±12.8	25.3±18.9
GGT (IU/L)	53.9±47.1	33.4±41.2*
T C (mg/dL)	206.9±29.5	205.3±32.6

*p<0.05

Table 4. Comparison of age and laboratory data between anti-HEV positive and negative residents in non hog raising area

	positive (N=16)	negative (N=363)
Age	58.7±10.7	60.6±9.6
AST(IU/L)	31.6±17.2	25.7±12.7†
ALT (IU/L)	31.5±23.3	24.9±13.6†
GGT (IU/L)	39.8±37.2	33.3±46.8
T C (mg/dL)	187.6±28.1	201.8±33.6†

† p<0.1

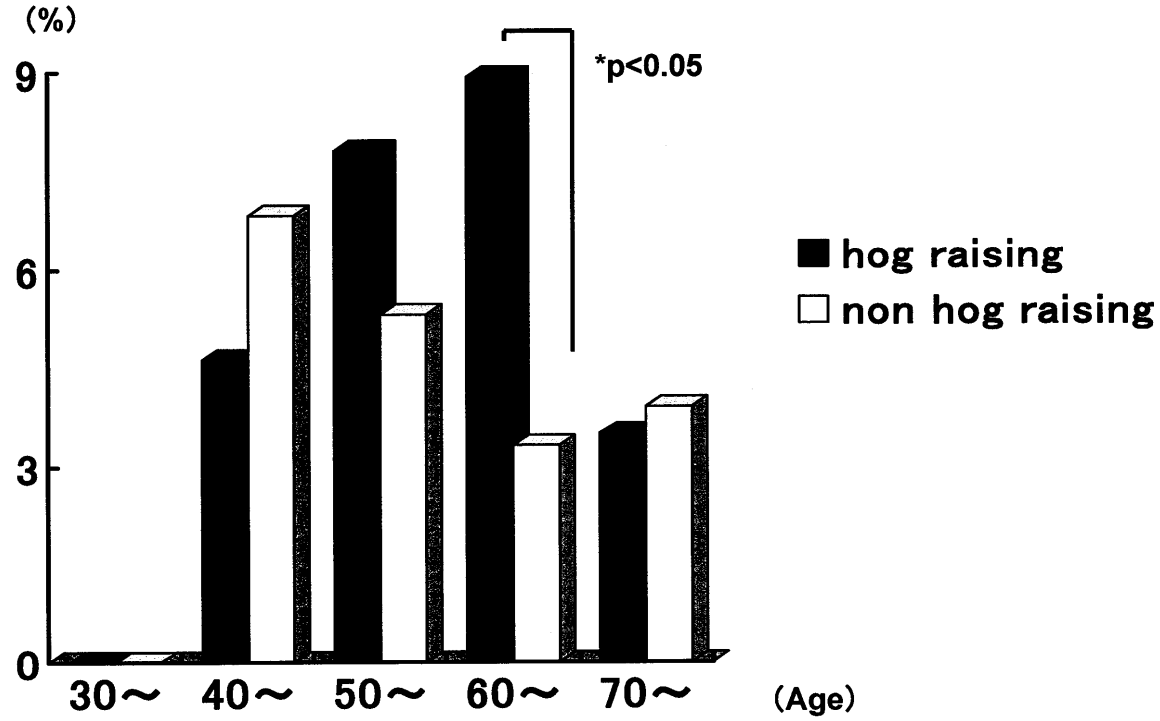

Figure 1. Age distribution of anti-HEV in hog raising and non hog raising area

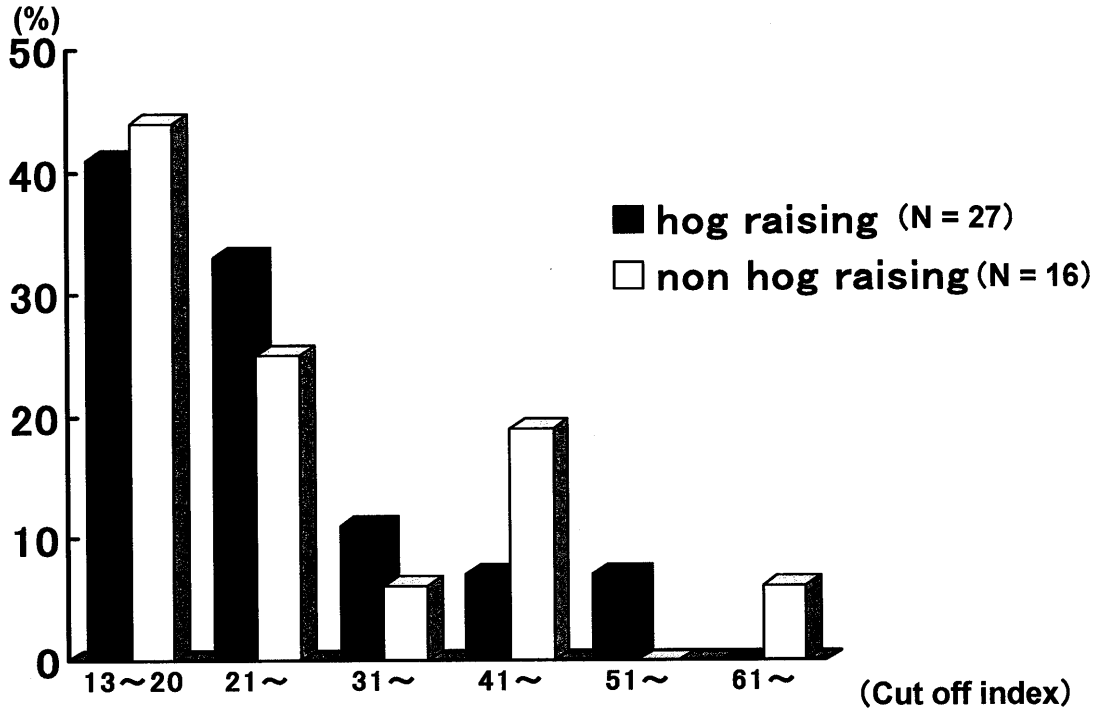

Figure 2. Distribution of anti-HEV titers in hog raising and non hog raising area

which occurred in individuals who traveled abroad in endemic countries after returning home[2]. However, new knowledge has been reported in succession that hepatitis E might be zoonosis which spreads via a food-borne mode among individuals in the community and the indigenous strain of HEV is the causative agent. Recent observations have revealed that those who ate raw meat of a swine, a wild boar or a deer were likely to be infected with HEV suggesting that these animals might have been an infection source for human beings, namely a reservoir of HEV[3-9].

A few studies on the relationship between residential neighborhoods of hoggeries or workers in hog raising business and hepatitis E virus infection have been reported so far, including those from Taiwan and United States[10-12]. They reported that high prevalence of anti-HEV was observed in workers in hog raising business compared with controls (27% vs. 8%, 11% vs. 2%, 51% vs. 25%, respectively). Our study is the first report that reviewed seroprevalence of anti-HEV in residents in hog raising area and non hog raising area. X prefedture is main stock-breeding prefecture having many hog raising business in our country. It is important to clarify the epidemiological status of HEV infection in the region such as X prefecture where a lot of hoggery is present for elucidation the route of HEV infection as a community acquired disease. It also will contribute to carrying out an administration policy including establishment of health guidance in the meat ingestion.

We found anti-HEV more commonly in hog raising area with increase of age than in non hog raising area (6.8% vs. 4.2%) and that anti-HEV positive rate showed a tendency to increase with aging in hog raising area and to decrease with aging in non hog raising area. Although the number of bleeding pigs has been increasing year by year, the number of hoggery has been decreasing under influence of modernization and colonization of the hog rising business simultaneously with by the rigidification of the laws about the raw sewage processing. This may contribute to the decrease of opportunity to be exposed to HEV in residents in hog raising area and it is also supposed that the environment surrounding hog raising business has changed compared with the time of the small scale breeding in the past. The same infection chance might have been present in the neighboring residents as for the hog raising practitioner in the times when sufficient raw sewage processing was not established and it is suggested that the hog raising business was partly responsible for the cause of HEV infection in neighboring inhabitants. On the other hand it is known that average day instar at the time of the swine slaughter for offering to meat in hog raising business is 200 days and until then all pigs were infected with HEV transiently13). Only pigs being still in the viremia of HEV at the time of slaughter have infectivity and could be a source of supply of HEV in the circulating market.

Therefore it is suggested that the difference may be present in a main route of HEV infection between hog raising area and non hog raising area, because we have found, in fact, that some residents positive for anti-HEV had a mild liver dysfunction and high anti-HEV titer in non hog raising area. We can not deny that these residents might have been in the recovery status from extreme recent HEV infection, for example, after consuming raw animal meat, because subclinical infection of HEV occurs more frequently than apparent infection1). Additionally, significant higher value of GGT in residents positive for anti-HEV in hog raising area may be caused by the fact that a lot of drinkers or obese persons were accidentally included in this group in the present study. These data will demonstrate that two main routes play a role in the transmission of HEV from animals to humans. One is when people living in the neighborhood of a hoggery ingest food or water contaminated with feces of

the swine. The other is when people, regardless of living area, eat raw or uncooked pork circulating in a market, home or restaurant. Therefore it is important to enforce the laws about the raw sewage processing and health guidance not to eat uncooked meat by government for preventing HEV infection not only to evade the risk of fulminant hepatitis but also posttransfusion by accidental blood donation [14,15].

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和文要旨

X県の地方自治体の中から、養豚業が存在しない非養豚地域の住民から379例(男女比132：247、平均年齢60.5歳)、養豚業が存在し飼育ブタ数2万頭以上の養豚地域の住民から395例(男女比131:264、平均年齢59.3歳)をそれぞれ任意に抽出し、IgG-HEV抗体(HEV抗体)をEIA法にて測定した。HEV抗体は非養豚地域で4.2%、養豚地域で6.8%に検出され、養豚地域に高率の傾向を認めた(p=0.11)。非養豚地域のHEV抗体陽性例および陰性例の平均ALT値(IU/L)はそれぞれ31.5±23.3 vs. 24.9±13.6、養豚地域のHEV抗体陽性例および陰性例の平均ALT値(IU/L)はそれぞれ30.1±12.8 vs. 25.3±18.9で、非養豚地域ではHEV抗体陽性例のALTが高値の傾向を認めた(p=0.069)。男女別HEV抗体陽性率は、非養豚地域5.3% vs. 3.6% 、養豚地域7.6% vs. 6.4%で差は認められなかった。年代別HEV抗体陽性率は、非養豚地域は加齢とともに漸減傾向、養豚地域では漸増傾向を示し、60歳代においては養豚地域が有意に高率であった(8.9 % vs. 3.3%, p<0.05)。またHEV抗体力価の分布は、非養豚地域では2峰性を、養豚地域では力価が高値になるにつれ減少を示した。これらのことから養豚業の存在と周辺住民のHEV感染との間には何らかの関連性が示唆され、非養豚地域と養豚地域の住民のHEVの感染様式には差が存在する可能性が示唆された。